Estimation of Agitation Intensity in the GI Tract in Humans and Dogs Based on in Vitro/in Vivo Correlation

Noriko Katori, Nobuo Aoyagi, and Tadao Terao

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In this study, we assessed the hydrodynamic flow around a dosage form in the GI tract in humans by comparing the characteristics of in vitro and in vivo release of two different types of controlled release acetaminophen (paracetamol) tablets, A and B. The former tablet showed an agitation speed-dependent release at a high speed range (50–100 rpm), whereas the latter showed this characteristic at a low speed range (10–50 rpm). The mean release amount-time profiles of tablets A and B in humans showed biphase characteristics, and the first phase of the absorption profiles of A and B was close to their in vitro profiles at a paddle speed of 10 rpm. The in vivo profiles were also superimposable on an in vitro dissolution curves obtained by the flow-through cell method at a flow rate of 1 mL/min (velocity 0.89 cm/min) or less. These results indicate that the hydrodynamic flow around the dosage forms in the human GI tract could be extremely low. The in vivo release rate of these tablets in dogs was greater than in humans, and was estimated to be equivalent to the release rate determined by the paddle method at 100 rpm. This indicates that a higher agitation intensity in the GI tract in dogs than in humans may be one cause of the discrepancies between humans and dogs in drug absorption studies.

KEY WORDS: absorption; dissolution tests; hydrodynamic flow in GI tract; controlled release; in vitro/in vivo relationship; acetaminophen.

INTRODUCTION

The development and evaluation of a formulation is facilitated if in vitro testing can predict in vivo performances. To establish a useful in vitro dissolution testing system for oral dosage forms, it is important to understand the fate of an administered dosage form, and in particular, the gastrointestinal (GI) factors affecting drug release. The major GI factors affecting dissolution properties include: pH, viscosity, and concentration of surfactant. The effects of GI fluid characteristics, especially pH, on dissolution have been extensively investigated. However, the effects of physical factors such as the hydrodynamic flow of GI fluid and the mechanical destructive forces due to GI motility on in vivo dissolution are poorly understood.

The present study was undertaken to clarify GI hydrodynamic conditions surrounding dosage forms, based on an in vitro-in vivo comparison of drug release properties. We prepared two different types of controlled release acetaminophen (paracetamol) tablets, both of which showed agitation speed-dependent release, one at a low speed range (10–50 rpm), and the other at a high speed range (50–100 rpm). According to previous results, we anticipated that these ranges would cover the agitation intensity in the human GI tract (1–5). The animal studies were carried out to reveal differences in GI agitation intensity between humans and dogs, since our previous studies (6) suggested that GI motility in dogs was stronger than in humans, possibly accounting for greater bioavailability in dogs of products with poor availability in humans.

MATERIALS AND METHODS

Dosage forms

Two types of controlled release tablets (8 mm diameter, 3 mm thick) of acetaminophen were prepared by direct compression. The components of the tablets are shown in Table I. The drug release rates were controlled by the dissolution characteristics of the components fumarate and tryptophan. The correct ratio of acidic (fumarate) and basic (tryptophan) excipients makes dissolution of the tablets independent of pH, and the side wall of the tablets was coated with ethylcellulose so that the dissolution properties were near zero order release, as previously reported (7). Acetaminophen (p-hydroxyacetanilide) and o-hydroxyacetanilide (internal standard for HPLC) were purchased from Tokyo Chemical Industry Co. Ltd. (Japan). All other reagents used were of analytical grade and available from commercial suppliers.

In vitro drug release

Dissolution tests were carried out at 37°C with 900–2000 mL of dissolution media, using the JP XII paddle, JP XII rotating basket (rotating speed fixed at 50 rpm), and USP flow-through cell (cell diameter 12 mm) methods. The rotating dialysis cell method (8) was also used, in which 5 mL of medium was placed in a cell made with HVLPM (Millipore® membrane) set at 15 hrpm horizontal rotation in 900 mL of dissolution medium at 37°C. For all dissolution tests, except for the study of pH effect, JP XII 2nd fluid (pH 6.8, for disintegration test) was used. The effects of pH and polysorbate 80 (0.01%) on drug release were investigated by the paddle method. The amount of drug dissolved was determined by the HPLC method described below, in which the sample solution was filtered and directly injected. All tests were carried out in duplicate or triplicate.

In vivo studies

Human Study

In the human study, the drug release amount and bioavailability parameters were calculated using the saliva concentration of acetaminophen; this has been reported to be proportional and virtually equivalent to serum drug concentration (9). Six healthy volunteers, five males and a female (age range, 30 to 52 years; weight, 50 to 67 kg) participated in the study after giving their written informed consent. The volunteers received a test tablet together with 200 mL of water in a crossover fashion according to a randomized
Table 1. Composition of Controlled Release Acetaminophen Tablets

<table>
<thead>
<tr>
<th>Component (mg)</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Fumarate</td>
<td>71.4</td>
<td>72.0</td>
</tr>
<tr>
<td>dl-Tryptophan</td>
<td>24.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td>2.6</td>
<td>—</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>2.0</td>
<td>—</td>
</tr>
<tr>
<td>Hydroxypropylcellulose</td>
<td>—</td>
<td>4.0</td>
</tr>
<tr>
<td>Total</td>
<td>200.0</td>
<td>200.0</td>
</tr>
</tbody>
</table>

block design. They were also given 100 mL of solution containing 100 mg of acetaminophen together with 100 mL of water, and there was at least a 7-day washout period between dosing. The volunteers were fasted from 10 h before to 4 h after the drug administration. Saliva samples were collected spontaneously for by each volunteer 1 to 2 min into a centrifuged tube for up to 24 h after dosing. The samples were frozen at −20 °C until analyzed.

Dog Study

Six male beagle dogs, weighing 12.0–15.5 kg, received a test tablet together with 30 mL of water in a crossover fashion according to a randomized block design, followed by i.v. and p.o. solution administration studies with a 7-day washout period between each dosing. The dogs were fasted for 12 h prior to and for 8 h after receiving the products. They were also given a 100-mg dose of acetaminophen solution orally and 50 mg of acetaminophen intravenously. Blood samples (3-mL) were taken for up to 24 h, and the plasma samples were kept frozen at −20 °C until assayed.

Analytical Methods

Acetaminophen was determined by high performance liquid chromatography at 240 nm using a column of Inertsil ODS-2 (150 × 4 mm, GL Science Inc., Japan) at 50 °C. The eluents used were: A, 1.5% acetic acid containing 15% methanol (for plasma sample); B, methanol-acetonitrile-0.05 M phosphate potassium buffer (pH 2.5) (8:7:85) mixture (for saliva and dissolution samples) at a flow rate of 1 mL/min.

Prior to analysis, the frozen samples were thawed out at room temperature and centrifuged at 3000 rpm for 20 min after vigorous mixing to remove precipitants of protein. To a 500-μL aliquot of saliva or plasma, 100 μL of 100 μg/mL o-hydroxyacetonilide internal standard solution and 5 mL of ethylacetate were added. The mixture was shaken for 15 min and then centrifuged at 2500 rpm for 5 min. A 4-μL aliquot of the supernatant was taken and dried under a stream of nitrogen at 50 °C. The residue was reconstituted with 200 μL of eluent, and a 50-μL volume was injected onto the column. Retention times of acetaminophen and o-hydroxyacetonilide were 4.7 and 9.9 min, respectively, for eluent A, and 4.0 and 8.0 min for eluent B. The average recovery value for acetaminophen was 94.3% from plasma and 103.3% from salvia.

The coefficients of variation of acetaminophen analysis varied from 0.5 to 5.3% over the range 1.0 to 50.0 μg/mL of acetaminophen, and the correlation coefficient of the standard curve was 0.999. The quantification limit for acetaminophen was about 100 ng/mL (CV 10%).

Data Analysis

Drug absorption in the dog following oral administration was calculated by point-area deconvolution (10) using i.v. administration data for weight function. When the i.v. data were used for weight function, the cumulative absorbed amount was normalized for the relative bioavailability of the p.o. solution to correct the first-pass metabolism effect in individual dogs. Because of the very fast absorption rate of acetaminophen from oral solution, the drug release profile from the slow release dosage form seemed to be reasonably reflected in the normalized absorption profile in the dog. The drug release in humans following oral administration was calculated by the constrained deconvolution method of Verotta et al (11), in which p.o. solution data were used for weight function. The weight function of deconvolution was determined by using the pharmacokinetic model parameters in all cases; these were fitted to a compartment model using the MULTI (12) program, in which Akaike’s information criterion was used for model selection.

RESULTS

In vitro dissolution test

The drug dissolution rate from tablet A was virtually unaffected by the agitation intensity at the range of 10–50 rpm paddle speed; however, it was slightly accelerated at 100 rpm (Fig. 1-A1). In contrast the dissolution rate of tablet B was greatly affected by agitation intensity at the range of 10–50 rpm paddle speed (Fig. 1-B1). The drug dissolution rate determined by the rotating dialysis cell method at 15 hrpm cell rotation was similar to that by the paddle method at 100 rpm for B, whereas, with A, the former exceeded the latter. The following observations suggested that A is more sensitive to destructive force: In the rotating dialysis cell method, the tablet is rolled in the membrane cell, and is gradually eroded from the surface due to friction between the tablet and membrane, which promotes dissolution. In contrast, in the paddle method, the tablet stays on the bottom of the dissolution apparatus without movement when the paddle speed is less than 100 rpm. The difference in dissolution rates between A and B could be due to differences in hydrophilicity between recipients. The difference in sensitivities for erosion between A and B could reflect the relationship between spontaneous dissolution rate (dissolution without destructive force) versus surface destruction rate. The two types of tablets tested did not disintegrate; however, the surface of the tablets would be gradually destroyed if there was a mechanical stress. If the spontaneous dissolution rate is large, as for B, destructive forces contribute little to promote drug release, whereas if the spontaneous dissolution rate of a tablet is relatively small, as for A, destructive forces contribute greatly to promote drug release.

In the flow-through cell method, the drug dissolution rates increased with increases in the flow rate of the dissolution medium (Fig. 1-A2, B2), although there was no difference in dissolution rates at flow rates of 1 and 2 mL/min (velocity of 0.89 and 1.76 cm/min). In the rotating basket